Comparison of a rapid immuno-chromatography assay with a standard ELISA for the detection of IgM and IgG antibodies against dengue viruses

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Received: 4 October 2017 / Accepted: 26 February 2018 / Published online: 17 April 2018
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Abstract A total of 765 blood samples collected from dengue suspected patients admitted to a Teaching Hospital in Sri Lanka were used to compare a rapid ICT assay with a standard ELISA for the detection of anti-dengue virus (DENV) IgM and IgG. Detection accuracy indices including sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), Chi square and Cohen's kappa values were determined for the ICT assay using the ELISA as a comparator for the detection of anti-DENV IgM and IgG. The rapid ICT assay showed a sensitivity of 64%, specificity of 75%, NPV of 68% and PPV of 72% for anti-DENV IgM detection. However, all the accuracy indices were relatively higher for the anti-DENV IgG detection by the ICT assay than those for anti-DENV IgM detection. Despite the low sensitivity for anti-DENV IgM detection by the ICT assay, considering the limitations in using ELISAs in resource limited regions, rapid ICT assays would be useful for the detection of more recent DENV infections. As many patients present after fever days 5 in the study area, anti-DENV IgM/IgG would be the suitable marker to be detected by rapid ICT assays in such areas.

Keywords Dengue · Rapid immuno-chromatography assays · ELISA · Anti-DENV IgM/IgG

The prevalence of clinically apparent dengue virus (DENV) infections has increased significantly in recent decades. Since there is no specific treatment for dengue, patients are solely managed by supportive therapy. Moreover, prevention of dengue is challenging for health authorities despite the exhausting efforts taken in the dengue endemic countries including Sri Lanka. Though the first recombinant tetravalent vaccine (Dengvaxia) registered for use in Mexico in 2015 [9], the prevention of dengue is mainly done using vector control strategies. With the increase in clinically apparent DENV infections, the early laboratory identification of DENV infection enhances better clinical monitoring during the early phase of the illness. On the other hand, detection of DENV infection will also contribute to accurate notifications to the authorities, enabling execution of prompt dengue control measures to the affected areas. Rapid immuno-chromatography (ICT) assays are commonly used to detect anti-DENV IgM/IgG for the identification of more recent DENV infections due to their rapidity and simplicity. As many seek medical care after fever days 5, anti-DENV IgM/IgG become the suitable marker to identify a recent DENV infection in many dengue endemic countries. Additionally, detecting the anti-DENV IgG together would help to differentiate primary and secondary DENV infections.

Rapid lateral flow ICT assays available for the qualitative detection of anti-DENV IgM, IgG, IgA and NS1 antigen in human blood require minimum time, technical expertise and infrastructure [1]. In Sri Lanka, laboratory diagnosis of DENV infection is done by detecting anti-

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DENV IgM and IgG using rapid ICT assays in many state and private laboratories. However, only a few studies have compared the ability of the rapid ICT assays to detect anti-DENV IgM and IgG with standard assays with better accuracy indices for anti-DENV IgM/IgG detection like ELISA. In this study we compared the ICT assay for anti-DENV IgM/IgG detection with a CDC recommended ELISA (Panbio Diagnostics, Australia).

A total of 765 blood samples collected between November 2009 and January 2012 from dengue suspected patients admitted to the Teaching Hospital, Jaffna (THJ) were tested using a rapid ICT assay (Cortez, USA) to detect anti-DENV IgM and IgG simultaneously. The same sera were tested for the presence of anti-DENV IgM and IgG using an anti-DENV IgM capture ELISA (Panbio Diagnostics, Australia) and anti-DENV IgG indirect ELISA (Panbio Diagnostics, Australia). Ethical approval for the study was obtained from the Ethics Review Committee of the Faculty of Medicine, University of Peradeniya, Sri Lanka (EC No.: 2010/EC/13).

For the anti-DENV IgM and IgG detection by the rapid ICT assay, 10 µl of serum sample was added to the sample window of the cassette followed by addition of 3–4 drops of diluent promptly to the reagent window of the cassette. The test results were read within 15–20 min. If the sample contained both anti-DENV IgM and IgG, 3 lines (anti-DENV IgM and IgG lines and the control line) were visible. If the sample contained only anti-DENV IgM or anti-DENV IgG only two lines were visible (anti-DENV IgM or IgG line and the control line) and if both antibodies were negative only the control line was visible.

For the anti-DENV IgM detection by ELISA, an aliquot (100 µl) of patient serum (diluted 1:100 in the diluent provided) was added to each well of the assay plate containing bound anti-human DENV IgM, which binds with human anti-DENV IgM in the patient's serum. The plate was incubated for 1 h at 37 °C. The wells in the assay plate were washed using the wash buffer. Then 100 µl of previously mixed antigen monoclonal antibody (Mab) tracer solution was added to the wells. The plate was incubated for 1 h at 37 °C and the wells were washed again using the wash buffer. The bound complexes were visualized after adding 100 µl of tetramethyl-benzidine substrate to the wells. After 10 min, the reaction was stopped by adding 100 µl of stop solution. The plates were read at 450 nm using a plate reader (Multiscan EX, Finland). The results were calculated and interpreted according to the manufacturer's instructions.

For the anti-DENV IgG detection by ELISA, an aliquot (100 µl) of patient serum (diluted 1:100 in the diluents provided) and the controls were added into their respective wells, which were pre-coated with DENV antigen mix (DENV serotypes 1, 2, 3 and 4). The DENV antigen mix

would bind with anti-DENV IgG present in the patient's serum. The plate was then incubated for 30 min at 37 °C. The wells in the assay plate were washed six times using the wash buffer. Then 100 µl of HRP conjugated anti-human IgG was added to the wells and the plate was incubated for 30 min at 37 °C. Wells were washed again using the wash buffer and the bound complexes were visualized after adding 100 µl of TMB substrate into the wells. After 10 min, the reaction was stopped by adding a 100 µl of stop solution. The plates were read at a wavelength of 450 nm with a reference wavelength of 600–650 nm and the results were interpreted according to the manufacturer's instructions.

Detection accuracy indices such as sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), Chi square and Cohen's kappa values were calculated using SPSS for Windows (Version 21.0; SPSS, Chicago, IL). The results from the rapid ICT assay for anti-DENV IgM and IgG detection were compared with that of ELISA (Table 1). Then the accuracy indices were determined by calculating the sensitivity, specificity, PPV and NPV of the rapid ICT assay for anti-DENV IgM and IgG detection (Table 2).

Of the 765 patients' sera tested, 343 and 383 were positive for anti-DENV IgM by the rapid ICT assay and ELISA, respectively. A total of 246 patients' sera were positive for anti-DENV IgM by both rapid ICT and ELISA. A total of 285 patients' sera were negative for anti-DENV IgM by both rapid ICT and ELISA. A total of 97 patients' sera positive for anti-DENV IgM by the rapid ICT assay was negative by the ELISA. A total of 137 patients' sera negative for anti-DENV IgM by the rapid ICT assay was positive by the ELISA (Table 1). Mean fever duration at the day of sample collection for testing was 5.5 ± 1.6 days.

Of the 765 sera tested, 427 and 460 were positive for anti-DENV IgG by the rapid ICT assay and ELISA, respectively. A total of 373 patients' sera were positive for anti-DENV IgG by both rapid ICT and ELISA. A total of 305 patients' sera were negative for anti-DENV IgG by both rapid ICT and ELISA. A total of 54 patients' sera positive for anti-DENV IgG by the rapid ICT assay was negative by ELISA. A total of 87 patients' sera negative for anti-DENV IgG by the rapid ICT assay was positive by ELISA (Table 1). The PPV for ICT assay for detecting anti-DENV IgG (87.4%) was greater than the ability of the ICT assay for detecting anti-DENV IgM (71.7%) (Table 2). We then compared the detection indices for patients with dengue fever (DF) and dengue haemorrhagic fever (DHF) (Table 2). There were no major differences between sensitivity and specificity of rapid ICT assay for the detection of anti-DENV IgM and IgG in patients with DF or DHF.

Table 1 Comparison of rapid ICT assay with the standard ELISA for anti-DENV IgM and IgG detection

ELISA	Rapid ICT assay Anti-DENV IgM		Rapid ICT assay Anti-DENV IgG	
	Positive	Negative	Positive	Negative
Anti-DENV IgM				
Positive	246 (32.1%)	137 (17.9%)	–	–
Negative	97 (12.7%)	285 (37.2%)	–	–
Anti-DENV IgG				
Positive	–	–	373 (48.7%)	87 (11.3%)
Negative	–	–	54 (7%)	251 (32.8%)

Table 2 Accuracy indices for the rapid ICT assay for anti-DENV IgM and IgG detection when compared to the ELISA

Rapid ICT assay	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Anti-DENV IgM	64.2	74.6	71.7	67.5
Anti-DENV IgG	81.1	82.3	87.4	74.3

PPV positive predictive value, NPV negative predictive value

A rapid and accurate diagnostic method to detect clinically apparent DENV infections is useful for managing dengue patients. Rapid ICT assays have been developed for the detection of anti-DENV IgM and IgG by a number of commercial manufacturers and these assays have been used widely due to the ease of use and rapid turnaround time. However, the detection capability of these assays for different viral markers varies in different geographical settings. Hence, there is a need to evaluate these ICT assays with a reference test for detecting clinically apparent DENV infections. A standard ELISA is commonly used as a comparator to validate rapid assays [2, 5, 7]. In this study, we compared a widely used rapid ICT assay (Cortez, USA) for its ability to detect anti-DENV IgM and IgG with a standard ELISA (Panbio Diagnostics, Australia).

Based on our study, detection of anti-DENV IgM by the rapid ICT assay showed a moderate sensitivity and NPV with a high specificity and PPV. Detection of anti-DENV IgG by the rapid ICT assay showed higher accuracy indices than those noted for anti-DENV IgM detection (Table 2). No significant difference was noted ($p > 0.05$) in the accuracy indices for anti-DENV IgM and IgG detection by the ICT assay between the DF and DHF patients (Table 3). A recent study conducted by Senaratne et al. in a different region of Sri Lanka tested only 119 samples for anti-DENV

IgM and IgG and incidentally this study also has used the same ELISA (Panbio Diagnostics, Australia) for validating a different ICT assay [8]. The sensitivity, specificity and the PPV of the ICT assay used by Senaratne et al. for anti-DENV IgM detection were higher than those observed in the current study indicating the differences in the accuracy indices of different ICT assays. Moreover, the difference between different ICT assays for detecting a particular marker using the same ELISA comparator indicates the importance of validating rapid ICT assays. Previous studies report a wide variation in the detection sensitivity and specificity ranging from 20 to 100% for the detection of anti-DENV IgM and anti-DENV IgG for some commercially available rapid ICT assays [2, 3, 5, 6, 8]. Moreover, sensitivity of anti-DENV IgM detection is significantly low in secondary infections [10] and thus the low sensitivity for anti-DENV IgM detection by the ICT assay in the current study is not surprising, as more than 60% of the study sample had secondary DENV infections. Combining DENV NS1 antigen with anti-DENV IgM or anti-DENV IgM/IgG in ICT assays might improve the detection sensitivity and specificity of more recent DENV infections considerably [3, 4, 7].

The ICT assays do not require any specialized equipment or training and the results are available within 25 min

Table 3 Accuracy indices for the rapid ICT assay for anti-DENV IgM and IgG detection in DF and DHF patients when compared to the ELISA

Clinical severity	Rapid ICT assay (%)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
DF (n = 285)	Anti-DENV IgM	63.6	81.7	71.1	71.3
	Anti-DENV IgG	77.8	83.6	87.9	75.8
DHF (n = 480)	Anti-DENV IgM	64.5	80.7	72	65.1
	Anti-DENV IgG	72.5	81.5	87	73.3

PPV positive predictive value, NPV negative predictive value

making them ideal for resource limited regions. The assay is relatively easy to perform and the interpretation of results is well defined. On the other hand, Blacksell, states that there may be inter-observer variations when ICT assays are used for the detection of common virological markers [1]. Methods such as virus isolation, viral nucleic acid detection (PCR) and ELISA need a specialized laboratory and well trained personnel and these are not usually available in most of the laboratories in resource limited regions. Considering the limitations of using a molecular/ELISA based diagnostic methods, rapid ICT assays are suited for resource limited regions. The rapid ICT assay costs around 4.2 US\$ per sample to detect anti-DENV IgM/IgG, however, the ELISA costs around 14.2 US\$ per sample to detect anti-DENV IgM/IgG and this shows the cost-effective diagnostic utility of ICT assays in resource limited regions. Rapid assays have the ability to detect and discriminate both anti-DENV IgM and IgG in a sample and they are able to differentiate primary from the secondary DENV infections [4]. However, some studies questioned the ability of the ICT to discriminate between primary and secondary DENV infections [6, 8].

In conclusion, the ICT assay showed low sensitivity for the detection of anti-DENV IgM, however, considering the limitations of ELISA for that purpose, the rapid ICT assay is useful for the detection of a more recent DENV infection. The ICT assay appears to be cost-effective when compared to the ELISA for anti-DENV IgM/IgG detection and thus is a valuable substitute to ELISA in small laboratories. The relatively high sensitivity and specificity of the rapid ICT assay for anti-DENV IgG detection suggest that DENV NS1 antigen and anti-DENV IgM/IgG in combination will enhance the detection of DENV infection in suspected patients. However, clinicians must be aware of the variation in the sensitivity, specificity, PPV and NPV of rapid ICT assays when interpreting data. It is better to select validated ICT assays in a given country and region with better accuracy indices for the detection of a dengue marker or markers.

Acknowledgements We thank the financial support provided from the Higher Education for the 21st Century (HETC) Grant (HETC JEN/O-MED/N7) from the Ministry of Higher Education, Sri Lanka

and Australian High Commission's Direct Aid Programme for ELISA kits.

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